

Mechanical properties of bioactive amorphous calcium phosphate/methacrylate composites

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ABSTRACT

Objectives. The aim of this study was to determine whether amorphous calcium phosphate (ACP)-containing composites, which have the ability to release mineralizing levels of Ca and PO₄ ions, have appropriate mechanical properties for use as base and lining materials.

Methods. Composites of pyrophosphate-stabilized ACP particulates (mass fraction of 40%) and photo-activated methacrylate resins (mass fraction of 60%) were tested for biaxial flexure strength (BFS), diametral tensile strength (DTS), and compressive strength (CS). Hydroxyapatite (HAP; mass fraction of 40%), and micro-sized glass (mass fraction of 50%) composites as well as a commercial visible light curable base/liner were also tested. The significance between mean values was determined by Student-Newman-Keuls multiple comparisons ($p < 0.05$).

Results. BFS of dry and wet (24 h at 37°C in water) ACP composites (60.3 and 62.0 MPa, respectively) were significantly lower than those of the comparison materials (79.2–109.3 MPa). CS values were likewise lower (62.9 MPa dry and 67.6 MPa wet vs 80.6–196.8 MPa) except for the wet base/liner (58.5 MPa). DTS of the dry ACP composite (21.8 MPa) was comparable with that of the HAP (22.8 MPa) and glass (25.5 MPa) composites, but lower than that of the base/liner (36.2 MPa). DTS decreased significantly when the ACP composite was wet (17.8 MPa).

Significance. These results suggest that the remineralizing ACP polymeric composites, although mechanically weaker in some respects than other polymeric composites, have properties suitable for use as base and lining materials. © 1998 Academy of Dental Materials. Published by Elsevier Science Ltd.

INTRODUCTION

Amorphous calcium phosphate (ACP), an important intermediate in hydroxyapatite (HAP) formation,

recently has been proposed for use as a bioactive additive in several Bis-GMA resin-based dental materials (Antonucci *et al.*, 1994, 1996; Skrtic *et al.*, 1995). Its potential as an additive for remineralization arises primarily because it is a single solid phase precursor to HAP, having biocompatibility with both hard and soft tissues, comparable with HAP and/or various di-, tri- and tetracalcium phosphates (Antonucci, 1986; Brown and Chow, 1986; Sugawara *et al.*, 1989; Fukase *et al.*, 1990; Antonucci *et al.*, 1991; LeGeros, 1991; Miyazaki *et al.*, 1993). Previous studies (Skrtic *et al.*, 1995) have shown that ACP-filled polymers are capable of releasing into aqueous media supersaturating levels of Ca²⁺ and PO₄ ions in proportions favorable for the formation of HAP. Composites containing P₂O₇⁴⁻-stabilized ACP, in particular, can sustain this release over extended periods of time. These composites have also been shown to be effective agents for remineralizing *in vitro* caries-like enamel lesions artificially induced in extracted bovine incisors (Skrtic *et al.*, 1995, 1996A). Based on these results, it has been proposed (Skrtic *et al.*, 1996a) that ACP-filled resins would make good Ca²⁺ and PO₄ ion-releasing materials for remineralization applications in dentistry.

ACP fillers, however, are deficient in their performance as bulk reinforcing agents in composite restorative applications when compared with commonly used silanized glass fillers (Antonucci, 1986). Therefore, their use would currently be more appropriate in applications where mechanical demands are less severe. For example, ACP may be useful as a prophylactic filler in BisGMA-based resins used as pit and fissure sealants, or as a reparative agent in base and lining materials.

TABLE 1: MONOMERS AND PHOTO INITIATOR SYSTEM COMPONENTS EMPLOYED FOR RESIN FORMULATIONS

Monomer	Manufacturer	Batch No.
2,2-bis[p-(2-hydroxy-3-methacryloxypropoxy) phenyl] propane	Bis-GMA Freeman Chemical Corp., Port Washington, WI	46-4005
Triethylene glycol dimethacrylate	TEGDMA Esschem, Essington, PA	9637446
2-hydroxyethyl methacrylate	HEMA Rohm Tech, Inc., Malden, MA	9687044
Zirconyl dimethacrylate	ZrM Rohm Tech, Inc., Malden, MA	D 1038
Camphorquinone	CQ Aldrich Chem. Co., Milwaukee, WI	n/a
Ethyl-4-N,N-dimethyl aminobenzoate	4EDMAB Aldrich Chem. Co., Milwaukee, WI	n/a

TABLE 2: RESIN COMPOSITION USED IN THE PREPARATION OF EXPERIMENTAL COMPOSITE SPECIMENS

	Bis-GMA	TEGDMA	HEMA	ZrM	CQ	4EDMAB
% by mass	35.1	35.1	28.0	0.8	0.2	0.8

In this study, the mechanical properties of a $P_2O_7^{4-}$ -stabilized ACP composite formulated with a hydrophilic Bis-GMA resin mixture was determined both before and after immersion in distilled water to establish whether it would have adequate mechanical strength for use as a base and lining material. For comparison, the properties of an HAP- and a glass-microfilled composite based on the same hydrophilic Bis-GMA resin were tested. In addition, a commercial visible light-curable base/liner also was evaluated in a similar fashion.

MATERIALS AND METHODS

Methacrylate resin formulations: The photo-activated resin was formulated from the commercial monomers and the photo initiator components listed in Table 1. The mass fraction composition of the resin is given in Table 2. All the experimental composites were prepared with this resin.

$P_2O_7^{4-}$ -stabilized ACP filler (Eanes *et al.*, 1965) was prepared by rapidly adding, while stirring, an equal volume of an 800 mmol/l $Ca(NO_3)_2$ solution to a 525 mmol/l Na_2HPO_4 /10.72 mmol/l $Na_4P_2O_7$ solution previously adjusted to pH 12.5 with 1 mol/l NaOH. The instantaneous precipitation reaction was carried out in a closed system under CO_2 -free N_2 at 22°C. In this way CO_2 adsorption by the precipitate was

minimized. After the pH stabilized at 10.5–11.0, which took less than 5 min, the suspension was filtered and the solid phase washed with ice-chilled ammoniated water and lyophilized. HAP filler was prepared by adding with stirring an equal volume of a 100 mmol/l $CaCl_2$ solution to a 60 mmol/l KH_2PO_4 solution at 22°C. Sufficient 2 mol/l KOH was then added to initiate precipitation and to stabilize the pH at 7.4 once the precipitation was completed. The suspension was stirred continuously overnight at 22°C under pH-stat control, filtered, washed and lyophilized.

The amorphous or apatitic state of the lyophilized solids was verified by X-ray diffraction (XRD). Patterns were recorded between 20 and 45° 2 θ on a powder diffractometer (Rigaku Denki Co. Ltd, Danvers, MA, USA) with graphite-monochromatized $CuK\alpha$ radiation ($\lambda = 1.5418$) generated at 40 kV and 25 mA. Previous TEM studies (Nylon *et al.*, 1972; Eanes *et al.*, 1973) have shown that dried ACP consists of powdery aggregates of spheroidal particles 20–200 nm in diameter.

An unsilanized, aggregated (average size = 40 nm) micro-sized glass filler (OX50; Degussa, Ridgefield, NJ, USA) was used to prepare the experimental microfilled glass composite.

Preparation and evaluation of the composite test specimens: Composite pastes were formulated by hand spatulation of the liquid resin and the various fillers (mass fraction of 40% ACP or HAP; mass fraction of 50% OX50). The homogenized pastes were kept under vacuum (2.7 kPa) overnight to eliminate air entrained during mixing. The handling properties of the ACP and the OX50 composite pastes were similar, probably because of the similarities in the morphology of their submicron aggregate fillers. For biaxial flexure strength (BFS) measurements, the pastes were pressed into Teflon cylindrical molds, 15.8–16.8 mm in diameter and 1.5–2.1 mm in thickness by using a plastic spatula to apply light mechanical pressure. For diametral tensile strength (DTS) measurements, the pastes were pressed into stainless steel cylindrical molds, 5.97–6 mm in diameter and 2.99–3.17 mm in height by applying light manual force. For compressive strength (CS) measurements, the pastes were pressed similarly into metal molds, 3.74–3.99 mm in diameter and 6.21–6.35 mm in height. Each opening of the various molds was then covered with a thin mylar film and a glass slide and the assemblies held together

with spring clips. The disks were polymerized by irradiating each face of the mold assembly for 2 min with visible light (TRIAD 2000 Densply/York Division, Densply International, York, PA, USA). After light-curing, the specimens were removed from their molds and either kept in air for 24 h at 37°C (dry samples) or in distilled water for 24 h at 37°C (wet samples).

Depth of cure (DC) was determined by measuring the thickness of cured composite along the direction of irradiation by using a slotted (2.67 mm × 3.3 mm × 11.0 mm) stainless steel rectangular mold with a removable stainless steel side. The uncured composite was packed into the mold and the top end was covered with a strip of mylar film. The composite was irradiated for 1 min with visible light (Max Lite, Caulk/Dentsply, Milford, DE, USA) through the top opening of the mold. During irradiation the wand of the light source was in contact with the mylar strip. After the uncured and partially cured portion was removed with a razor blade, the cured portion of the composite was removed from the mold, and the dimension parallel to the irradiation direction was measured to the nearest 0.01 mm with a micrometer. The value of this dimension was taken as the depth of cure.

The mechanical strengths of the various composite disk specimens were determined with and without aqueous immersion after 24 h storage at 37°C. BFS, DTS, and CS were determined by using a computer-controlled universal testing machine (Instron 5500R, Instron, Canton, MA, USA) operated by Instron Merlin Software Series 9.

For BFS measurements the strength of a thin disk specimen, placed flat on a lower support circle of three stainless steel balls, was determined under a static load. The failure stress was calculated according to the following equation (Wachtman *et al.*, 1972; Kirstein and Woley, 1967):

$$BFS = AL/t^2 \quad (1)$$

where $A = -[3/4\pi(X - Y)]$, $X = (1 + \nu)\ln(r_1/r_s)^2 + [(1 - \nu)/2](r_1/r_s)^2$, $Y = (1 + \nu)[1 + \ln(r_{sc}/r_s)^2]$, and where ν = Poisson's ratio, r_1 = radius of the piston applying the load at the surface of contact, r_{sc} = radius of the support circle, r_s = radius of disk specimen, L = applied load at failure, and t = thickness of disk specimen.

The DTS values of the various composites were determined according to ANSI/ADA specification No. 27 (ANSI/ADA, 1977). Thus, for DTS measurements, the compressive load was placed by a flat plate against the side of the cylindrical disk specimen. The crosshead speed of the computer-controlled universal testing machine was 10 mm/min. CS was measured by dividing the maximum load in compression on the ends of the cylindrical specimens by the original cross-sectional area of the test specimens. The compression load was applied at a crosshead speed of 1 mm/min. Test specimens of the commercial visible light-curable base/liner (Timeline, Caulk/Dentsply, Milford, DE, USA) also were prepared for mechanical strength and the depth of cure evaluation.

Results were statistically examined by analysis of

variance (ANOVA). Measurements obtained from dry specimens were analyzed separately from those obtained after immersion. The significance of the observed difference between mean values was evaluated by Student-Newman-Keuls multiple comparisons ($p < 0.05$). The significance ($p < 0.05$) of the difference between the dry vs the wet strength value of each of the four materials tested was determined using the two-sided Student *t*-test.

RESULTS

The mean biaxial flexure strength (BFS) values of both dry and wet ACP composites were significantly lower than the corresponding mean values of HAP- and OX50 glass-filled composites and of the Timeline base/liner after 24 h storage in air (Table 3). No significant change occurred in the mean BFS values after each of the four materials was immersed in distilled water for 24 h. The mean diametral tensile strength (DTS) value of the dry ACP composite was comparable with those of the HAP- and OX50 glass-filled composites, although lower than the base/liner (Table 3). The mean DTS value of the ACP composite decreased significantly (21.9 MPa vs 17.8 MPa) when immersed in water for 24 h. This latter value was significantly lower than those of the other materials following immersion. The mean compressive strength (CS) values of both dry and wet ACP composite were statistically comparable with those of the HAP composite and the base/liner but were significantly lower than that of the OX50 glass-filled composite (Table 3). Pairwise comparison of the dry vs wet ACP-, HAP-, and OX50-composites indicated that no significant difference existed in the mean CS value after each of these three composites were stored for 24 h in water. However, after 24 h exposure to water the CS of the base/liner deteriorated significantly. The mean depth of cure (DC) value of the ACP composite was significantly greater than the mean DC values of the HAP composite and the base/liner but less than the mean DC value of the OX50 composite (Table 4).

DISCUSSION

Although not as strong in CS (63–68 MPa) as zinc phosphate cements (130–160 MPa) or hybrid glass ionomer cements (96–126 MPa), the data show that ACP composites have DTS greater than the former and about the same as the latter (Craig, 1997). In addition, the data in the present study indicate that the loss of the mineral filler by diffusion of Ca^{2+} and PO_4 ions from these composites on exposure to aqueous environments may not necessarily result in a significant deterioration of their mechanical properties, especially BFS and CS. A possible mechanism for retaining strength under these mass loss conditions may involve some internal conversion of ACP into HAP (Skrtec *et al.*, 1995). Our data show that composites made with HAP are stronger than those containing ACP. Moreover, despite this internal conversion of ACP into HAP, an earlier study (Skrtec *et al.*, 1995) showed that sufficient unconverted ACP remains to provide sustained release of Ca^{2+} and PO_4 ions.

TABLE 3: BIAxIAL FLEXURE STRENGTH (BFS), DIAMETRAL TENSILE STRENGTH (DTS) AND COMPRESSIVE STRENGTH (CS) OF DRY AND WET ACP-, HAP- AND OX50 GLASS-FILLED COMPOSITES AND TIMELINE BASE/LINER

	Dry	Wet
BFS (MPa)		
ACP-filled	60.3 ± 8.5 [12]	62.0 ± 12.0 [15]
HAP-filled	85.6 ± 23.4 [27] ^B	79.2 ± 21.7 [14] ^A
OX50 glass-filled	85.0 ± 21.9 [9] ^B	109.3 ± 39.5 [6] ^C
Timeline base/liner	94.4 ± 13.1 [6] ^B	89.8 ± 17.1 [6] ^A
DTS (MPa)		
ACP-filled	21.9 ± 3.7 [26]	17.8 ± 2.2 [37]
HAP-filled	22.8 ± 5.0 [21]	20.6 ± 3.4 [21] ^B
OX50 glass-filled	25.5 ± 4.5 [10]	25.1 ± 4.6 [20] ^C
Timeline base/liner	36.2 ± 1.4 [5] ^C	28.8 ± 3.6 [7] ^C
CS (MPa)		
ACP-filled	62.9 ± 20.8 [18]	67.6 ± 21.2 [13]
HAP-filled	83.0 ± 28.6 [15]	93.1 ± 39.3 [12]
OX50 glass-filled	185.0 ± 30.8 [9] ^C	196.8 ± 43.2 [20] ^C
Timeline base/liner	80.6 ± 6.4 [5]	58.5 ± 6.0 [5]

TABLE 4: DEPTH OF CURE (DC) FOR ACP-, HAP-, OX50 GLASS-FILLED COMPOSITES AND TIMELINE BASE/LINER

	DC (mm)
ACP-filled	6.64 ± 0.40 [13] ^A
HAP-filled	4.98 ± 0.37 [15] ^A
OX50 glass-filled	8.95 ± 0.45 [11] ^A
Timeline base/liner	3.18 ± 0.07 [6] ^A

Therefore, the results from the present study indicate that ACP composites have mechanical properties that are probably adequate for application as base/liner materials and possibly as sealants as well. The unique ability of ACP composites to release Ca^{2+} and PO_4 ions coupled with compressive strengths that exceed those of conventional self-curing calcium hydroxide cements (e.g., CS = 12–26 MPa) would seem to indicate a role for these remineralizing composites as cavity liners and bases (Craig, 1997). In this capacity, they could promote the formation of reparative dentin, encourage recovery of injured pulp, and protect the pulp against chemical and thermal insults. A potential advantage of ACP-containing sealant resins is that their unique ability to release Ca^{2+} and PO_4 ions in a sustained manner could augment the ability of the sealant to protect teeth from pit and fissure decay. For this application wear resistance may need to be assessed. Recently, it has been shown that ACP-containing composites can be made stronger by modifying ACP fillers with glass-forming agents (Skrtec *et al.*, 1996b).

In summary, the data show that remineralizing ACP composites of the type described in this study, have adequate mechanical strength for use as base and lining materials.

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